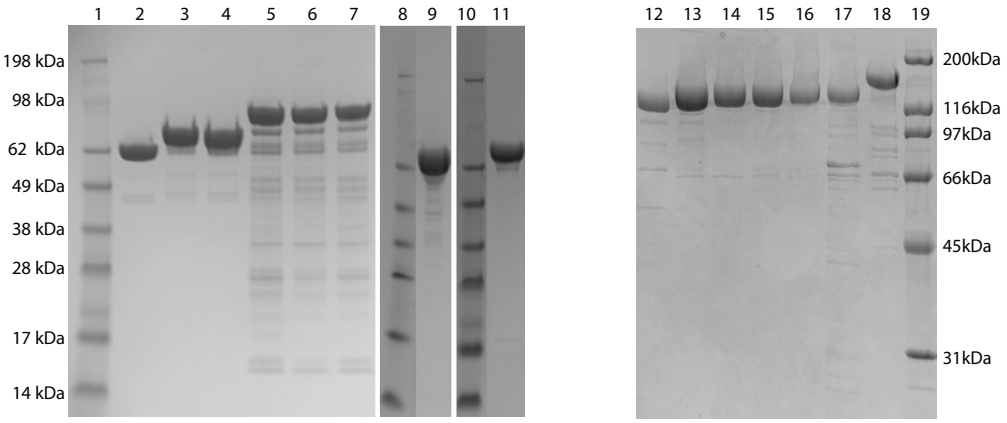
Figure S1

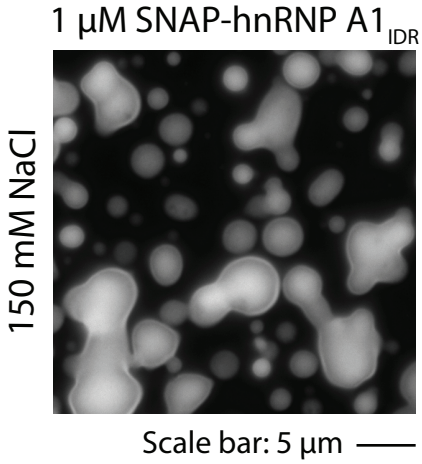
A



1. Ladder
2. MBP-SNAP-His6
3. MBP-SNAP-Lsm4$_{IDR}$-His6
4. MBP-SNAP-Pub1$_{IDR}$-His6
5. His6-MBP-SNAP-hnRNP A1$_{WT}$
6. His6-MBP-SNAP-hnRNP A1$_{D262}$
7. His6-MBP-SNAP-hnRNP A1$_{\Delta Hexa}$
8. Ladder
9. MBP-SNAP-eIF4GII$_{IDR}$-His6
10. Ladder
11. MBP-SNAP-hnRNP A1$_{IDR}$-His6

12. MBP-SNAP-PTB-His6
13. MBP-SNAP-PTB-Pub1$_{IDR}$-His6
14. MBP-SNAP-PTB-Lsm4$_{IDR}$-His6
15. MBP-SNAP-PTB-eIF4GII$_{IDR}$-His6
16. MBP-SNAP-PTB-Tia11$_{IDR}$-His6
17. MBP-SNAP-PTB-hnRNPA1$_{IDR}$-His6
18. MBP-SNAP-PTB-FUS$_{IDR}$-His6
19. Ladder

B



Supplemental Figure Legends

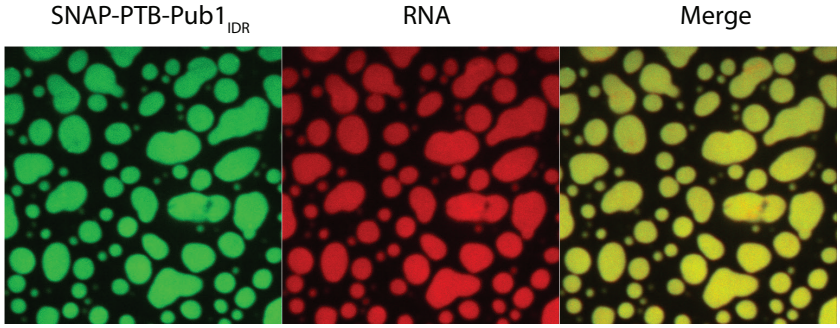
Figure S1. Crowding agent promotes the LLPS of hnRNPA1$_{IDR}$. Related to Figure 1.

(A) Coomassie blue stained SDS-PAGE gels of purified proteins used in this study.

(B) Fluorescent microscopy images of SNAP-Surface 488 labeled SNAP-hnRNPA1$_{IDR}$ forming droplets at low protein concentrations in the presence of 100 mg/ml PEG 3350.

Figure S2

A


SNAP-PTB-Pub1IDR
RNA
Merge

B

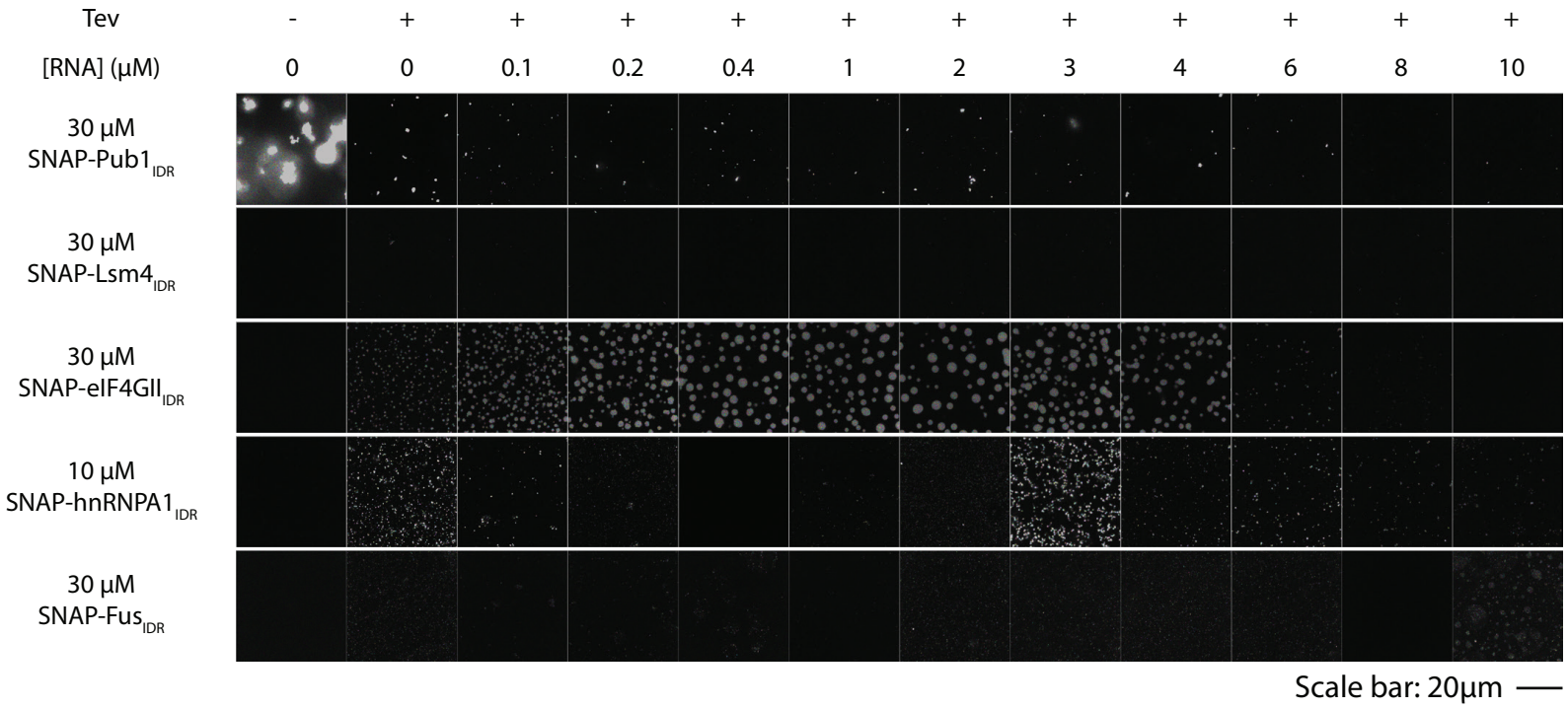
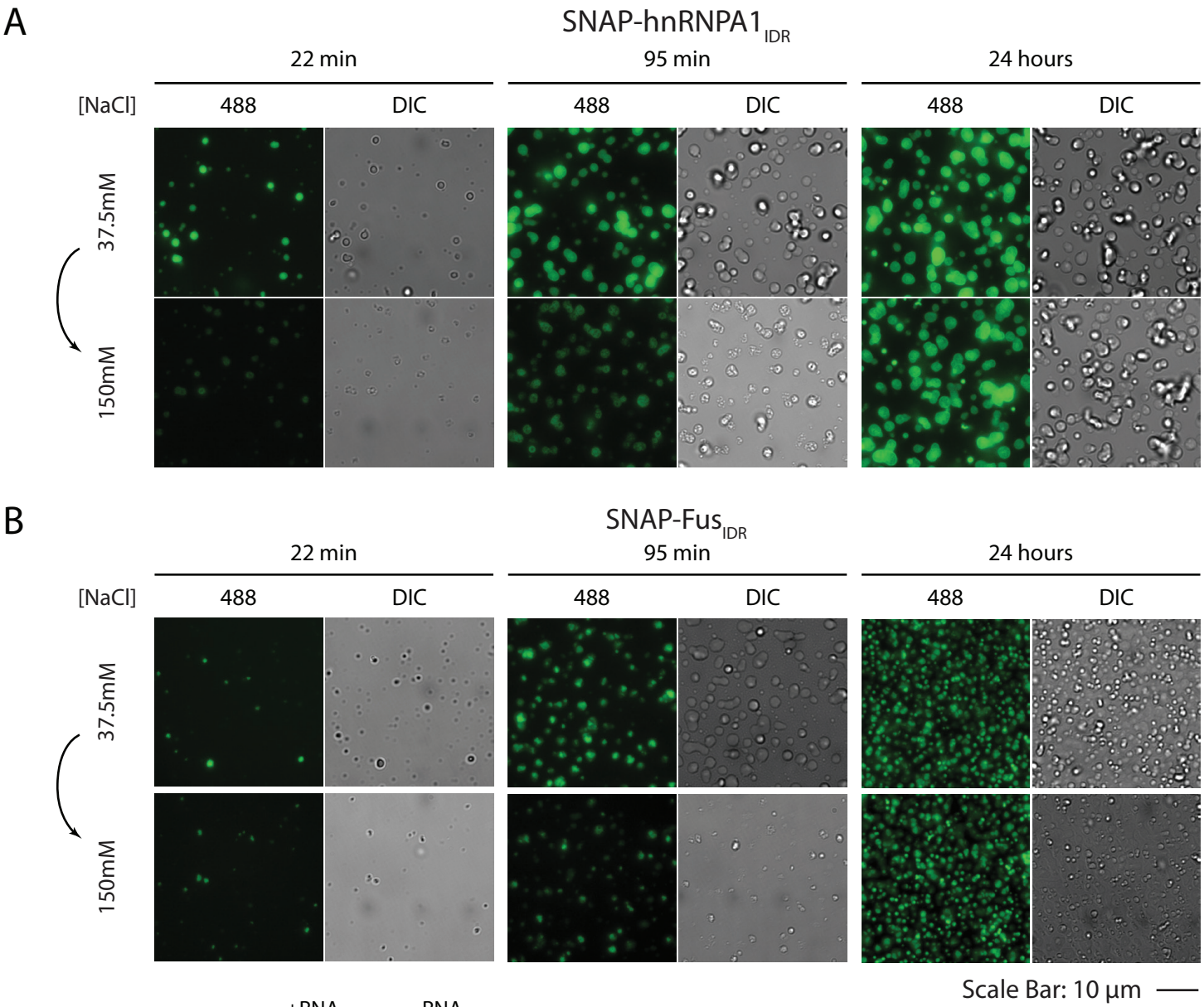

Tev
- + + + + + + + + + + +
[RNA] (μM)
0 0 0.1 0.2 0.4 1 2 3 4 6 8 10
30 μM SNAP-Pub1IDR
30 μM SNAP-Lsm4IDR
30 μM SNAP-eIF4GIIIDR
10 μM SNAP-hnRNPA1IDR
30 μM SNAP-FusIDR
Scale bar: 20μm

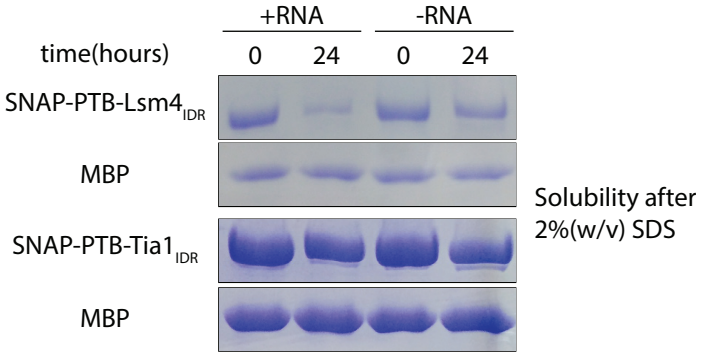
Figure S2. RNA Does Not Stimulate LLPS of Most SNAP-IDR Proteins lacking PTB. Related to Figure 2.

(A) Cy3 labeled RNA (0.5 μM, red) and SNAP-PTB-Pub1$_{IDR}$ (3 μM, green) co-localize within liquid droplets at 100 mM NaCl.

(B) Fluorescence microscopy images of SNAP-IDRs (at the indicated concentrations) titrated with RNA. Proteins are labeled with SNAP-Surface 649.

Figure S3





D

	SNAP-PTB-Pub1$_{IDR}$ RNA	SNAP-PTB-eIF4GII$_{IDR}$ RNA
$I_{labeled}$ (A.U.)	4183	7246
$C_{labeled}$ (μM)	1.228	2.082
Unlabeled/Labeled	300	150
$C_{unlabeled}$ (μM)	368.4	312.3

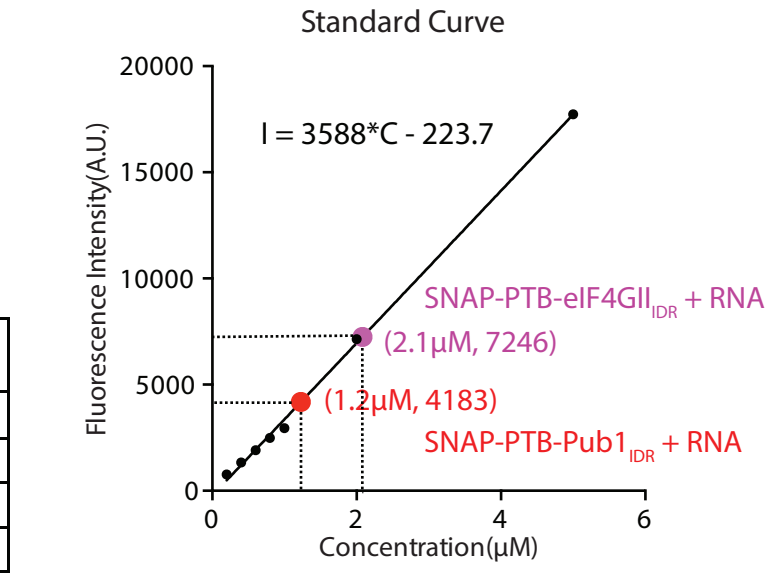


Figure S3. Phase Separated Droplets of SNAP-IDRs Mature over Time. Related to Figure 3.

(A) Fluorescence microscopy images of structures formed by SNAP-hnRNPA1$_{IDR}$ (4 μM) at 37.5 mM NaCl. At indicated time points, NaCl was raised to 150 mM total concentration and structures that remained were imaged. Both fluorescent and DIC images were taken for better illustration. Images at each time point are shown with the same intensity scale.

(B) Fluorescence microscopy images of structures formed by SNAP-Fus$_{IDR}$ (10.75 μM) at 37.5 mM NaCl. At indicated time points, NaCl was raised to 150 mM total concentration and structures that remained were imaged. Both fluorescent and DIC images were taken for better illustration. Images at each time point are shown in the same intensity scale.

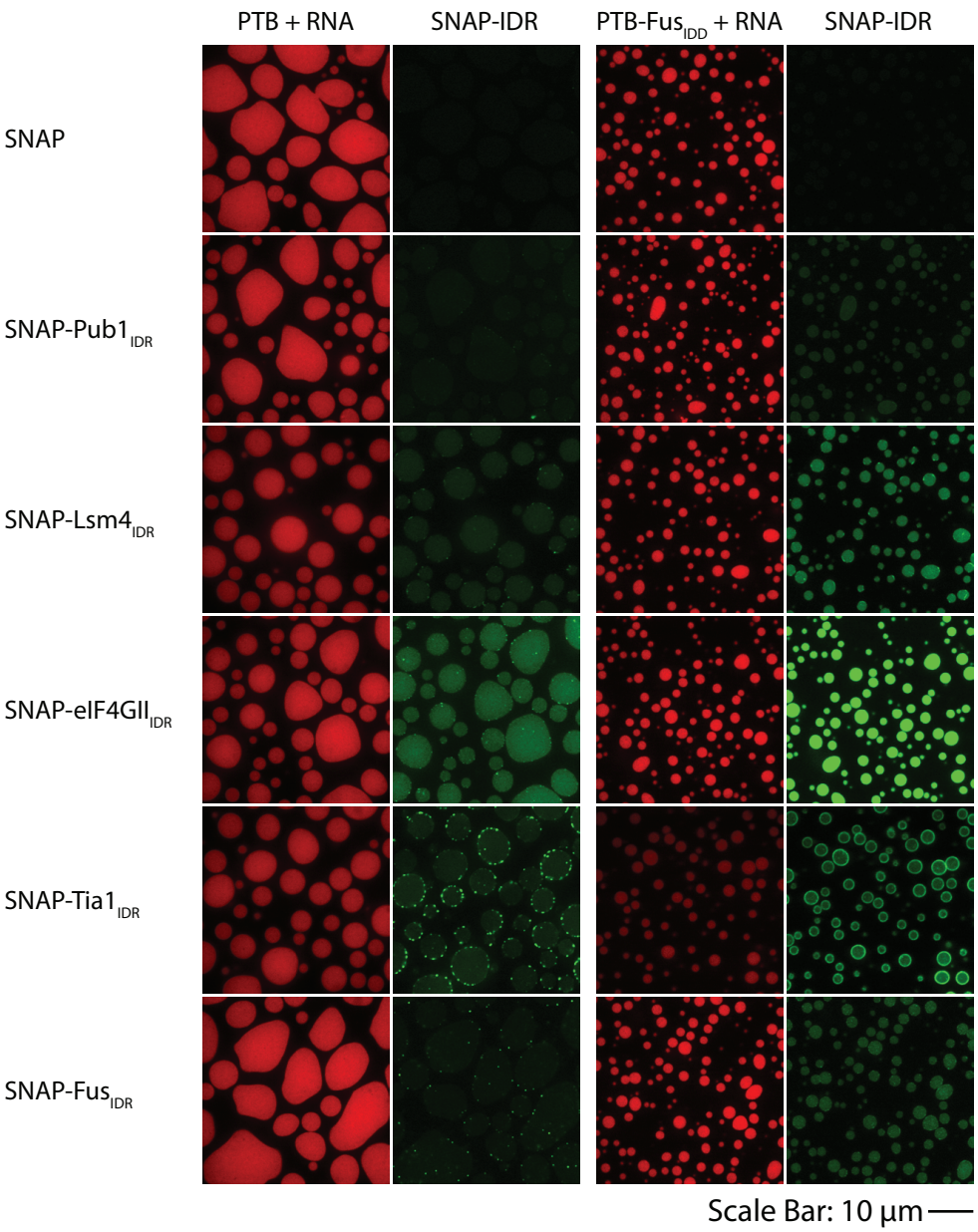
(C) SDS-PAGE assays of the amount of SDS-soluble species present at different time points after the initiation of phase separation by addition of RNA. MBP-SNAP-PTB-Lsm4$_{IDR}$ (5 μM) or MBP-SNAP-PTB-TIA1$_{IDR}$ (5 μM) were mixed with RNA (1.6 μM) (phase separation) or buffer (no phase separation) and TEV protease. At the indicated times SDS was added to 2%(w/v) followed by 5 minutes of centrifugation and filtration through an 0.22 um filter. The supernatant was then loaded into the gel and stained with Coomassie Blue. TEV protease was present to remove MBP, which serves as an internal loading control.

(D) The absolute concentrations of the fluorophore-labeled proteins within the liquid droplets were calculated from the droplet intensities according to the standard curve of a series of pure fluorophores at different concentrations. Droplets of SNAP-PTB-Pub1$_{IDR}$ (7.5 μM) plus RNA (2.4 μM) were doped with 25 nM fluorophore-labeled SNAP-PTB-Pub1$_{IDD}$; Droplets of SNAP-PTB-eIF4GII$_{IDR}$ (3.75 μM) plus RNA (1.2 μM) were doped with 25 nM fluorophore-labeled SNAP-PTB-eIF4GII$_{IDR}$. The proteins were labeled with SNAP-Surface 649.

(E) The summary of the absolute concentrations of proteins within the droplets of SNAP-PTB-Pub1$_{IDR}$ plus RNA and SNAP-PTB-eIF4GII$_{IDR}$ plus RNA.

Figure S4

A


PTB + RNA
SNAP-IDR
PTB-Fus$_{IDD}$ + RNA
SNAP-IDR
SNAP
SNAP-Pub1$_{IDR}$
SNAP-Lsm4$_{IDR}$
SNAP-eIF4GII$_{IDR}$
SNAP-Tia1$_{IDR}$
SNAP-Fus$_{IDR}$
Scale Bar: 10 µm

B

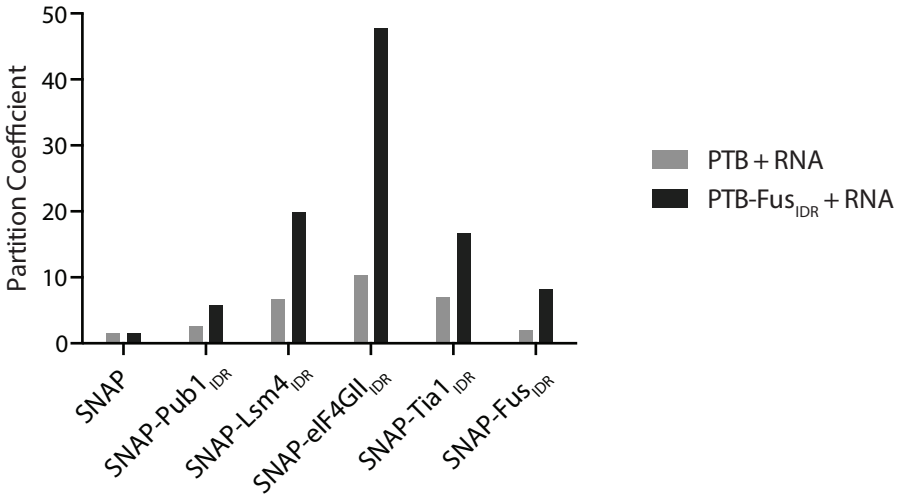

Partition Coefficient
50
40
30
20
10
0
SNAP
SNAP-Pub1$_{IDR}$
SNAP-Lsm4$_{IDR}$
SNAP-eIF4GII$_{IDR}$
SNAP-Tia1$_{IDR}$
SNAP-Fus$_{IDR}$
PTB + RNA
PTB-Fus$_{IDR}$ + RNA

Figure S4. IDR Dependent Phase Separated Droplets Recruit SNAP-IDR Probes. Related to Figure 4.

(A) Representative images showing the partitioning of SNAP-IDR probes (100 nM, labeled with SNAP-Surface 488, green) into the liquid droplets (red) of PTB or PTB-FUS_{IDR} plus Cy3-labeled RNA.

(B) Quantification of the SNAP-IDR partition coefficients in experiments from panel A.

Figure S5

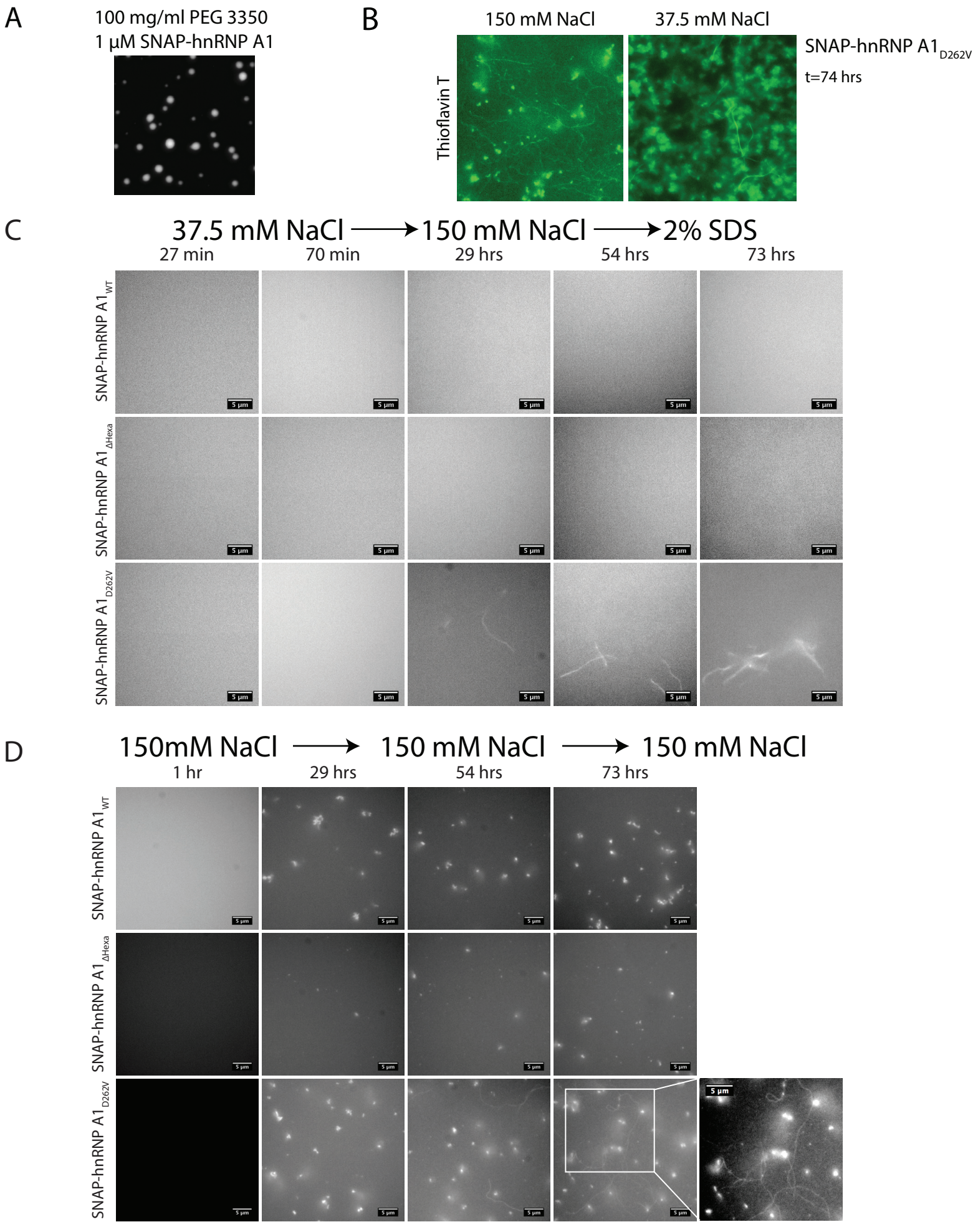

A
100 mg/ml PEG 3350
1 µM SNAP-hnRNP A1
B
150 mM NaCl
37.5 mM NaCl
Thioflavin T
SNAP-hnRNP A1D262V
t=74 hrs
C
37.5 mM NaCl → 150 mM NaCl → 2% SDS
27 min
70 min
29 hrs
54 hrs
73 hrs
SNAP-hnRNP A1WT
SNAP-hnRNP A1ΔHexa
SNAP-hnRNP A1D262V
5 µm
D
150mM NaCl → 150 mM NaCl → 150 mM NaCl
1 hr
29 hrs
54 hrs
73 hrs
SNAP-hnRNP A1WT
SNAP-hnRNP A1ΔHexa
SNAP-hnRNP A1D262V
5 µm

Figure S5. hnRNPA1$_{D262V}$ Mutant Forms SDS Resistant Fibers. Related to Figure 5.

(A) Fluorescent microscopy images of SNAP-Surface 488 labeled SNAP-hnRNP A1$_{WT}$ forming droplets at low protein concentrations in the presence of 100mg/ml PEG 3350.

(B) Fluorescence microscopy images of SNAP-hnRNP A1$_{WT}$ (25 μM) forming Thioflavin T-stained fibers. Fibers were observed in both low salt (37.5 mM NaCl) and high salt (150 mM NaCl) after incubation.

(C) Fluorescence microscopy images of SDS-resistant structures formed by SNAP-hnRNPA1$_{WT}$, SNAP-hnRNPA1$_{\Delta Hexa}$ and SNAP-hnRNPA1$_{D262V}$ (all 25 μM) present at different time points after initiation of phase separation at 37.5 mM NaCl. At the indicated time points NaCl was raised to a final concentration of 150 mM and SDS was then added to a final concentration of 2 % (w/v). The structures that remained were imaged.

(D) Fluorescence microscopy images of structures formed by SNAP-hnRNPA1$_{WT}$, SNAP-hnRNPA1$_{\Delta Hexa}$ and SNAP-hnRNPA1$_{D262V}$ (all 25 μM) after incubation for the indicated time at 150 mM NaCl.

Figure S6

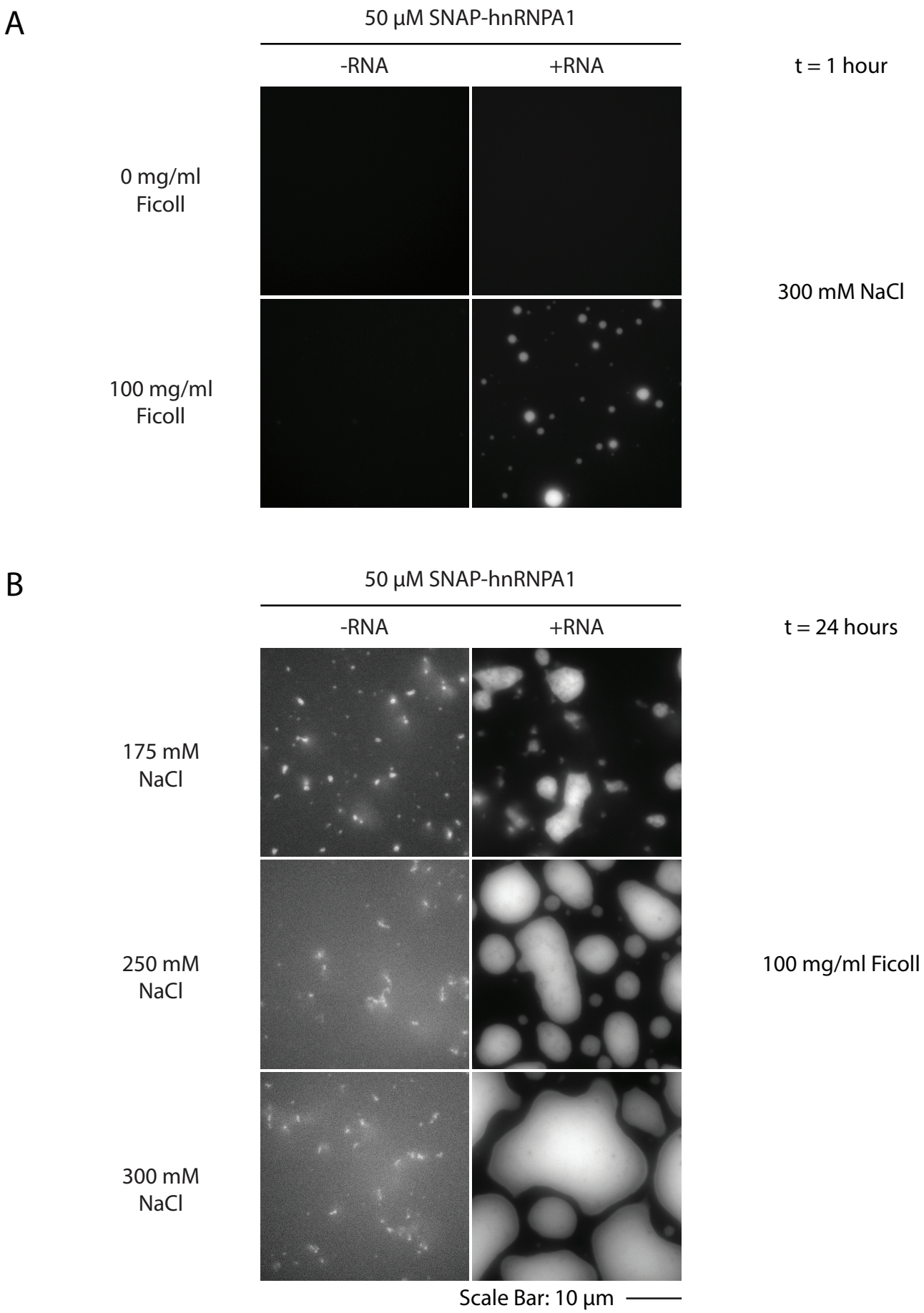

A
50 µM SNAP-hnRNPA1
-RNA
+RNA
t = 1 hour
0 mg/ml Ficoll
100 mg/ml Ficoll
300 mM NaCl
B
50 µM SNAP-hnRNPA1
-RNA
+RNA
t = 24 hours
175 mM NaCl
250 mM NaCl
300 mM NaCl
100 mg/ml Ficoll
Scale Bar: 10 µm

Figure S6. Both RNA and Ficoll Promote LLPS of Full-Length hnRNPA1. Related to Figure 6.

(A) Fluorescence microscopy images of SNAP-hnRNPA1 (50 μM) with or without RNA (100 μM) in the presence or absence of 100mg/ml Ficoll at 300mM NaCl.

(B) Fluorescence microscopy images of SNAP-hnRNPA1 (50 μM) with or without RNA(100μM) at indicated salt concentrations. 100mg/ml Ficoll.

Movie S1. Droplets of SNAP-hnRNPA1$_{IDR}$ (6.9 μM) at the Salt Concentration of 37.5 mM merged. Related to Figure 1.

Movie S2. Droplets of SNAP-Fus$_{IDR}$ (10.75 μM) at the Salt Concentration of 37.5 mM Merged. Related to Figure 1.

Movie S3. Droplets of SNAP-PTB-Pub1$_{IDR}$ and RNA at the Salt Concentration of 100 mM Merged. Related to Figure 2.

Movie S4. Droplets of SNAP-hnRNPA1 (50 μM) at the Salt Concentration of 37.5 mM Merged. Related to Figure 5.

The buffer contains 100mM NaCl, 100mg/ml Ficoll 400 as crowding reagents.

Table S1

	Time(hr)	N	t1/2(sec)	Ratio of recovery
SNAP -PTB -$Pub1_{IDR}$ RNA	1	5	29.8 ± 1.2	0.829 ± 0.011
	14	5	342.3 ± 28.3	0.751 ± 0.045
	24	3	N.A.	N.A.
SNAP -PTB -$Lsm4_{IDR}$ RNA	1	3	34.6 ± 4.0	0.668 ± 0.033
	14	2	N.A.	N.A.
SNAP -PTB -$eIF4GII_{IDR}$ RNA	1	5	57.9 ± 2.3	0.778 ± 0.021
	24	4	64.4 ± 5.3	0.226 ± 0.005
SNAP -PTB -$Tia1_{IDR}$ RNA	1	5	63.5 ± 4.2	0.348 ± 0.015
	14	2	N.A.	N.A.
SNAP -PTB -Fus_{IDR} RNA	1	3	19.0 ± 0.9	0.855 ± 0.013
	14	3	274.0 ± 7.3	0.726 ± 0.038
	24	2	337.0 ± 7.5	0.584 ± 0.070
	48	2	621.5 ± 228.2	0.538 ± 0.151

Mean ± SEM

Table S1. Statistics of FRAP Experiments on Droplets of SNAP-PTB-IDRs and RNA. Related to Figure 3.

The data are shown as mean ± SEM. N, the number of replicates.

Table S2

Name in paper	Protein	Uniprot Entry	Organism	Position(s)
MBP	MBP	P0AEX9	*Escherichia coli*	27-392
U1A	U1A	P09012	*Homo sapiens*	1-95
PTB	PTB	P26599	*Homo sapiens*	1-531
GFP	EGFP	C5MKY7	*Aequorea victoria*	1-239, A207K, L222K, F224K
$Pub1_{IDR}$	Pub1	P32588	*Saccharomyces cerevisiae*	243-327
$Lsm4_{IDR}$	Lsm4	P40070	*Saccharomyces cerevisiae*	91-187
$eIF4GII_{IDR}$	eIF4GII	P39936	*Saccharomyces cerevisiae*	13-97
$TIA1_{IDR}$	TIA1	P31483	*Homo sapiens*	290-386
Fus_{IDR}	Fus	P35637	*Homo sapiens*	1-237
$hnRNPA1_{IDR}$	hnRNPA1	P09651-2	*Homo sapiens*	186-320
hnRNPA1	hnRNPA1	P09651-2	*Homo sapiens*	1-320
$hnRNPA1_{\Delta IDR}$	hnRNPA1	P09651-2	*Homo sapiens*	1-185
$hnRNPA1_{\Delta Hexa}$	hnRNPA1	P09651-2	*Homo sapiens*	1-286, 293-320
$hnRNPA1_{D262V}$	hnRNPA1	P09651-2	*Homo sapiens*	1-320, D262V

RNA	Sequence
PTB_ligand	$(UCUCUAAAAA)_5$
hnRNPA1_ligand	UAGGGACUUAGGGUUCUCUCUAGGGACUUAGGGUUCUCUCUAGGGA

Table S2. Proteins and RNAs Used in this Study. Related to Experimental Procedures.

Supplemental Experimental Procedures

Materials

The MTTH (MBP-Tev-Tev-His6) vector was engineered by inserting a TEV cleavage site after MBP and a C-terminal TEV-cleavable His6 tag right before the stop codon on the basis of pMal-c2 (NEB). For the constructs of SNAP-PTB-IDRs, SNAP tag (NEB) was cloned into the vector after N-terminal TEV cleavage site using EcoRI and SalI, for the recombinant proteins capable of organic fluorophore conjugation. A GGSKGGSE linker was inserted after SNAP tag. PTB was then cloned using SalI and AgeI and IDRs were cloned after PTB using AgeI and BamHI. For the constructs of SNAP-IDRs, everything is the same as SNAP-PTB-IDRs, except that PTB was omitted. For the constructs of EGFP-IDRs, EGFP was cloned after N-terminal TEV cleavage site using EcoRI and AgeI and IDRs were cloned after EGFP using AgeI and BamHI. The monomeric mutations (A207K, L222K, F224K) were made for EGFP. All the constructs were verified by DNA sequencing. For the construct of PTB-Fus_{IDR}, PTB and FUS_{1-214} were cloned into MTTH using NdeI and BamHI, with a GGSAAAGGS linker between them. For the construct of PTB, PTB was cloned using NdeI and BamHI into a pet11a based vector in which N-terminal TEV cleavage site was present. For full length hnRNPA1, hnRNPA1 was cloned into a pet11a-based vector, which was engineered to contain N-terminal PreScission-cleavable His6-MBP-SNAP tag, using Kpn1 and Not1.

All the RNAs were synthesized by Integrated DNA Technologies, Inc., Iowa. See also Table S2.

Protein Purification

For SNAP-PTB-IDRs and EGFP-IDRs, the proteins were expressed in *E. coli* strain $BL21(DE3)T1^{R}$ (Sigma-Aldrich). Bacteria were grown in LB at 37 °C and induced at OD600 0.6-1.0 with 1 mM IPTG at 18 °C for 16 hours. Cells were lysed by homogenization (EmulsiFlex-C5, Avestin) and followed by centrifugation at 50,000g for 30 min at 4 °C. Since these proteins have both N-terminal Tev-cleavable MBP tag

and a C-terminal Tev-cleavable His6 tag for affinity purification, they were purified through a tandem of Ni-NTA column (Qiagen) and Amylose column (NEB). The proteins were then purified using a Superdex 200 column equilibrated in the buffer of 100 mM NaCl, 20 mM imidazole, pH 7.0, 1 mM DTT, 10% glycerol. The proteins were flash-frozen and stored at -80˚C. All the assays for these proteins (except where indicated otherwise) were performed in the same buffer.

SNAP-IDR and SNAP-hnRNPA1 constructs were expressed in *E. coli* BL21 (DE3) (Agilent Technologies). Cells were grown in TB at 37˚C and induced at OD600 0.8-1.2 with 1 mM IPTG. Cells were harvested after 3 hours growth at 37˚C. Cells were lysed by homogenization (EmulsiFlex- C3, Avestin). Lysates were cleared by centrifugation at 25,661g for 20 min at 4˚C. The proteins were purified by nickel affinity chromatography (Fisher HisPur) and were dialyzed overnight at 4˚C against 150 mM NaCl, 20 mM Tris pH 7.4 and 1mM DTT. SNAP-IDRs were then further purified by amylose affinity chromotaography (NEB Amylose Resin) and eluted in 150 mM NaCl, 20 mM Tris 7.4, 40 mM maltose, 5 mM BME. Amylose purification was followed by overnight dialysis at 4˚C against 150 mM NaCl, 20 mM Tris pH 7.4 and 1 mM DTT. Glycerol was added to 20% for cryoprotection prior to flash-freezing and storage at -80˚C.

Proteins were concentrated via centrifugal filtration (Amicon Ultra Centrifugal Filter Unit, 10K MWCO). An additional ultra-centrifugation (Beckman TLA 100.2, 50,000 RPM, 30', 4˚C) step was applied to remove potential aggregates prior to droplet assembly experiments. The concentrations of all the proteins were measured from the absorbance at 280 nm on Nanodrop and the extinction coefficients were obtained from ExPASy ProtParam (http://web.expasy.org/protparam/).

Fluorescent Labeling of Proteins

The SNAP-tag containing proteins were labeled with either SNAP-Surface 649 or SNAP-Surface 488 (NEB). SNAP-hnRNPA1 constructs had N-terminal purification

tags removed by the addition of HRV C3 Protease (EMD Millipore HRV C3 Protease) during dye conjugation, while SNAP-IDRs had their tags removed at a later step (described in **Droplet Assembly**). By following the manufacturer's protocol, after 0.5-2 hours incubation at room temperature, the unreacted substrate was removed by Zeba spin desalting column, 7K MWCO (Thermo Scientific). The concentration of the labeled protein was then quantified by the absorbance at 655 nm(250,000 $M^{-1}cm^{-1}$, SNAP Surface 649) or 506 nm(90,000 $M^{-1}cm^{-1}$, SNAP Surface 488).

Droplet Assembly

Droplet Assembly for SNAP-IDRs and SNAP-hnRNPA1

The proteins were diluted to the indicated concentration with the buffer of 20 mM Tris pH 7.4, 1 mM DTT to reach the final salt concentration of 37.5 mM NaCl while maintaining pH and DTT concentration. 2% of the proteins were labeled with SNAP fluorophores. Reactions were placed in passivated glass-bottomed 96 well plates (PerkinsElmer Glass-Bottomed ViewPlate, coated with 3%BSA for15 minutes and then washed with H_2O three times) and sealed with PCR plate sealant tape to minimize evaporation during the experimental time. While the N-terminal purification tags of His6-MBP-SNAP-hnRNPA1$_{FL}$ were removed by HRV C3 protease during dye conjugation step, the N-terminal MBP tag and C-terminal His6 tag of MBP-SNAP-IDRs were cleaved off in the reaction by the addition of Tev protease (Promega ProTEV). For Thioflavin T staining experiments, 25 μM Thioflavin T (Abcam) was added in the solution and imaged with the Deltavision FITC filterset.

Droplet Assembly for SNAP-PTB-IDRs

Proteins (5 μM) and RNA (1.6 μM) were mixed with the indicated concentration in the buffer of 100 mM NaCl, 20 mM imidazole, pH 7.0, 1 mM DTT, 10% glycerol (and 100 mg/ml BSA when indicated). TEV protease was always present in the samples to remove N-terminal MBP tag and C-terminal His6 tag. 100 nM proteins labeled with SNAP-Surface 649 were used to visualize the liquid droplets (if there is any) under fluorescence microscopy. The reaction was placed in a glass-bottom chamber, which was coated with 3% BSA and washed three times with H_2O. For Thiovlavin T

staining experiments, 10 μM Thioflavin T was added in the solution and excited with 445 nm laser.

Fluorescence Microscopy

All images of SNAP-IDRs and SNAP-hnRNPA1 were acquired on a DeltaVision epi-fluoresence microscope, equipped with a SCMOS camera and LED illumination. The EGFP-IDR recruitment assay was performed on a Nikon AR1 LSM confocal microscope. All images of SNAP-PTB-IDRs were acquired on a Leica-based spinning disk confocal microscope (EMCCD digital camera, ImagEM X2, Hamamatsu; confocal scanner unit, CSU-X1, Yokogawa).

Phase Diagram

SNAP-PTB and SNAP-PTB-FUS_{IDR} were mixed with RNA in the same way described in **Droplet Assembly**. Phase separation was evaluated using spining disk confocal microscope mentioned above with a 100x objective lens after overnight incubation.

Fluorescence Recovery After Photobleaching

Fluorescence recovery after photobleaching (FRAP) experiments were performed on the same spinning disk confocal microscope mentioned above. The SNAP-tag containing proteins were labeled with SNAP-Surface 649 and bleached with full laser power for 30 iterations using a 405 nm laser line. The droplets were > 5 μm in diameter and the bleaching area was ~ 2 μm in diameter. Time-lapse images were acquired at 637 nm. Images were processed in ImageJ. Background intensity was first subtracted. Then the image of a homogenous solution containing 800nM SNAP-Surface 649 fluorophore (without phase separation) was obtained under the same illumination condition. The maximum intensity of this image was first measured and used to divide the whole image. The resulting image was used to divide all the images captured later for correction of uneven illumination. At each time point, the averaged fluorescence intensity within the bleaching spot was also divided by the intensity of a neighboring unbleached area of the same size to correct for changes in illumination during the time course of imaging. The corrected intensities within the

bleaching spot during recovery were fit to a single exponential growth curve to yield the half time and the ratio of recovery ($[I_{max}-I_{min}]/[I_0-I_{min}]$; $I_{max,}$ the plateau intensity obtained from the fitting; I_{min}, the intensity at t = 0; $I_{0,}$ the intensity before photobleaching) using GraphPad Prism 5 (GraphPad Software), as shown in Table S1. Data are reported as mean ± SEM, n ≥ 2. For recovery curve, points are shown as mean ± SD.

SDS-PAGE Assay for High-salt and SDS Soluble Species in Droplets

Identical samples containing 5 μM MBP-SNAP-PTB-$Lsm4_{IDR}$ (or 5 μM MBP-SNAP-PTB-$Tia1_{IDR}$) plus 1.6 μM RNA(phase separation) or buffer(no phase separation) were prepared. Tev protease was present to cleave off MBP, which remained soluble and thus served as an internal loading control. At the indicated time points, NaCl was raised to 500 mM total concentration to disassemble the droplets by abolishing the interaction between proteins and RNA. After one-minute incubation followed by centrifugation at 15,000g for 5 minutes, the supernatant was carefully removed and loaded on an SDS-PAGE gel. Gels were stained with Coomassie blue. The pellet was examined by TEM (see below). Alternatively, 2% (w/v) SDS was added at the indicated time points to disassemble the droplets. After incubation for 10 minutes followed by centrifugation at 15,000g for 5 minutes, the supernatant was further passed through 0.22 μm filter and the flowthrough was loaded on an SDS-PAGE gel. Gels were stained with Coomassie blue.

For SNAP-$hnRNPA1_{WT}$, SNAP-$hnRNPA1_{\Delta Hexa}$, SNAP-$hnRNPA1_{D262V}$, 25 μM proteins were first treated with HRV C3 at room temperature for 5 hours to cleave off MBP. To initiate phase separation the proteins were diluted to a final salt concentration of 37.5 mM NaCl. At indicated time points, NaCl was raised to a final concentration of 150 mM. After incubation for 5 minutes followed by centrifugation at 15,000g for 5 minutes, the supernatant was carefully removed and loaded on an SDS-PAGE gel. Gels were stained with Coomassie blue. The intensities of the bands were measured in ImageJ and normalized to the intensities of the internal control, MBP. The intensities relative to time point 0 hour were plotted.

Transmission Electron Microscopy

The pellet after high salt wash (see previous section) at 24 hours was resuspended in buffer (100 mM NaCl, 20 mM imidazole pH 7.0, 1 mM DTT) by brief sonication and directly transferred to a TEM grid (FCF300-Cu grid, Electron Microscopy Sciences) and stained with a 5 µl drop of 1% (w/v) PTA (phosphotungstic acid, pH adjusted to 8.0 with NaOH) for 1 minute. After the removal of the PTA solution, the grid was air-dried. The images were obtained on FEI Tecnai transmission electron microscope.

IDD Recruitment Assay

Partitioning of EGFP-IDRs into Droplets of PTB or PTB-Fus_{IDR} Plus RNA

Droplets were formed by either 10 µM PTB plus 3.2 µM RNA (note that PTB phase separates at lower concentrations than SNAP-PTB) or 1.25 µM PTB-Fus_{IDR} plus 0.4 µM RNA. In both cases, droplets were labeled with 10 nM 3'-Cy3 RNA and 100 nM GFP-IDR was used as probe. TEV protease was present to remove MBP tag. After 1 hour incubation, images were acquired using the spinning disk confocal microscope mentioned above at 637 nm and 561 nm simultaneously. Background intensity was subtracted and an image of a homogeneous solution was used to correct for uneven illumination. Droplet intensities were measured by averaging the intensities at the center (with diameter 2.5 µm smaller than that of the droplets) of droplets (~50-100 total number) from at least three different areas. For bulk intensities, identical samples were prepared in microcentrifuge tubes for 1 hour and centrifuged at 21,130g for 5 minutes. The supernatants were transferred to the same glass-bottom chamber and the intensities were measured in the same way. Intensities were converted to concentrations through a GFP calibration curve. The partition coefficient is defined as $[GFP]_{droplet}$ / $[GFP]_{bulk}$, and shown as mean ± SD from three measurements each of which averaged all the droplets across four random slide regions. P values were obtained using unpaired t-test. A representative image for each condition was chosen and the same brightness and contrast were used to show the relative intensities.

Partitioning of EGFP-IDRs into Droplets of SNAP-hnRNPA1

Fluorescently tagged 30 μM SNAP-hnRNPA1 (SNAP-Surface 649) with purification tags removed was mixed with 100 nM GFP-IDR or GFP alone in the presence of TEV protease (Promega ProTEV) to remove the N and C terminal purification tags from GFP constructs. Samples were incubated for 30 minutes at room temperature to allow for cleavage of purification tags prior to droplet assembly. Samples were diluted with 20 mM Tris pH 7.4 to indicated protein and salt concentrations. Of each reaction 3 aliquots were plated into passivated glass-bottomed 96 well plates (3% (w/v) BSA, 15 minutes, washed 3 times with H_2O; PerkinsElmer Glass-Bottomed ViewPlate). After 45 minutes the other half was spun at 16,300g for 3 minutes, and three aliquots of the supernatant were removed to the 96 well plate. Droplets and supernatant wells were imaged with different instrument parameters due to the small dynamic range of the Nikon AR1 LSM Confocal microscope used. Small Fus aggregates were excluded from the droplet region. The reported relative enrichment is defined as $I_{droplet}$ / $I_{bulk.}$

The Determination of the Absolute Concentration of Proteins within Droplets

The droplets were formed by either 7.5 μM SNAP-PTB-Pub1$_{IDR}$ + 2.4 μM RNA + 25 nM SNAP-Surface 649 labeled SNAP-PTB-Pub1$_{IDR}$ or 3.75 μM SNAP-PTB-eIF4GII$_{IDR}$ + 1.2 μM RNA + 25nM SNAP-Surface 649 labeled SNAP-PTB-eIF4GII$_{IDR.}$ After 1 hour incubation, the images of droplets were taken in the spinning disk confocal microscopy mentioned above. Background intensity was subtracted and uneven illumination was corrected with a homogenous fluorescent solution. The intensities at the center of the droplets were counted as the droplet intensities, which were converted into absolute concentrations of labeled proteins within droplets. Since the ratio between the labeled and unlabeled protein was known, the absolute concentration of proteins within droplets were obtained.